REMARKS

Claims 1 – 12 and 18 are pending in the application. Claims 10 and 13-17 have been cancelled. Claim 18 has been amended. No new claims have been added. No new matter has been added.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

Claim Rejections Withdrawn

The Examiner has withdrawn the rejection to the claims under 35 USC §112, second paragraph.

The Examiner has withdrawn the rejection to claims 1 – 9 and 11 - 12 under 35 USC §112, first paragraph.

Claim Rejections 35 USC §112

The Examiner has maintained the rejection to claim 18 under 35 USC §112, first paragraph. The Examiner argues that the specification, "while being enabled for a method of reducing or inhibiting invasiveness and metastasis of tumor cells expressing Gb3, does not reasonably provide enablement for the prevention of invasiveness and metastasis of tumor cells." (Office Action, p.2). Applicants respectfully disagree.

Without acquiescing to any validity of the Examiner's rejection, Applicants have amended the claims.

Claim 18, as amended, is directed to a method of reducing, or inhibiting invasiveness and metastasis of colon tumor cells in a subject, wherein the tumor cells produce Gb3 comprising administering to the subject a therapeutically effective amount of a B-subunit of Shiga toxin.

Accordingly, Applicants request that the rejection be withdrawn.

Claim Rejections 35 USC §103(a)

The Examiner has maintained the rejection to claims 1 -12 and 18 as being unpatentable over the combination of LaCasse et al.(Blood vol. 88 p.1561 (1995)) in view of Marcato et al. (Infection and Immunity vol. 70 p.1279 (3.2002) and Strockbine et al. (J Bacteriology vol. 170 p.1116), Accession Number 2002:397002, Green (US 2002/0081307) and Applicant's admission on page 6, lines 1 – 2 of the specification. Applicants respectfully disagree.

The claims recite a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a B-subunit of Shiga toxin.

The present invention is based on the discovery that glycosphingolipid (GSL) globotriaosylceramide (Gb3) is a marker for potentially invasive and metastatic human colon cancer cells, and that Gb3 expression turns non-invasive epithelial cells into invasive ones, and that Shiga toxin 1 B-subunit can selectively kill tumor cells.

In the specification, Applicants teach that Shiga toxin represents a broad class of so-called AB5 bacterial toxins, and that Applicants have **chosen Shiga toxin 1 B-subunit** for use in the invention as claimed. At page 6, beginning at line 30, Applicants teach that there are a number of Shiga toxin variants and subunits:

> The sequences of numerous Shiga toxin variants and subunits are known in the art. For example, the Shiga toxin 1 B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400300 and 32400303, the Shiga toxin 2 B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No. 13359150, the Shiga toxin 1 A-subunit is set from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400299 and 32400302, and the Shiga toxin 2 A-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No.15718405.

Further, at page 7, beginning at line 23, Applicants teach that different cell types have been shown to respond differently to B-subunit treatment:

> B-subunit alone in much higher concentrations than holotoxin triggers apoptosis in BL and HEp-2 cells...while THP-1 cells, which bind Stx1, are insensitive to B-subunit treatment (Mangeney M, Lingwood CA, Taga S, Caillou B, Tursz T, Wiels J. 1993. Apoptosis induced in Burkitts lymphoma cells via GB3/CD77, a glycolipid antigen. Cancer Res. 53: 5314-5319).

The LaCasse reference does not teach or suggest all the limitations of the instant claims. In particular, the LaCasse reference does not teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a B-subunit of Shiga toxin.

The LaCasse reference is directed to the use of shiga like toxin (SLT-1) in human bone marrow (BM) purging. LaCasse uses Shiga Like Toxin (SLT-1) which "kills cells by inhibiting protein synthesis." (p.1561). The purpose of the study described by LaCasse "was to establish the potential of a natural toxin (SLT-1) in purging B-cell lymphomas from BM." (p.1563). LaCasse reference is directed only to the use of SLT-1 in BM purging and provides no teaching or suggestion for the use of SLT-1 in reducing, or inhibiting **invasiveness and metastasis of tumor cells in a subject**.

The Examiner has indicated that the LaCasse reference teaches "treatment of human B cell lymphoma using Shiga toxin 1." (Office Action, p.7). The Examiner argues that LaCasse "also discloses that the toxin was administered after the cancer is present." (Office Action, p.6). The Examiner further argues that "on page 6 of the specification, applicant admits the toxins are known to bind to Gb3 expressing cells, therefore it is expected that the cells of the reference are Gb3 expressing cells." (Office Action, p.7). The Examiner admits that LaCasse et al. "do not disclose the use of the B unit of Shiga toxin 1 or 2" and that "Marcato et al discloses that it is the B subunit of the toxins (either Shiga toxin 1 or 2) that are responsible for the toxicity." (Office Action, p.7).

None of the Marcato, Strockbine or Greene references cure the defects of the LaCasse reference. None of the references, alone or in combination, teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a

subject; wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a B-subunit of Shiga toxin.

Nowhere in the Marcato reference is there teaching or suggestion of a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a B-subunit of Shiga toxin as claimed.

The Examiner argues that Applicant has argued each reference separately. The Examiner argues that "Marcato was cited to show why one skilled in the art would cho(o)se the subunit." (Office Action, p.4 – 5).

The Examiner argues that "it would have been obvious that one skilled in the art can use the B subunit from either toxin and expect the same results and thus it would have been obvious to one of ordinary skill in the art to use the B subunit of either toxin in the treatment of the primary reference with the expected benefit of treating B cell lymphoma." Office Action of 5/23/2008, p.8).

The Marcato reference is directed to use of the cloned shiga toxin B (Stx2 B) subunit to induce apoptosis in Burkitt Lymphoma B-cells. Nowhere does Marcato teach inhibiting invasiveness and metastasis of tumor cells in a subject. Further, the Marcato reference teaches that "unlike the two holotoxins, Stx2 B subunit mediated apoptosis does not involve inhibition of protein biosynthesis." (Abstract, p.1279). This is different from the teachings of Lacasse, where SLT-1 kills cells by inhibiting protein synthesis.

One of skill in the art would not be motivated to use StxB, taught by Marcato, in place of SLT-1, taught by LaCasse.

Therefore, the teachings of the cited art, when combined, do not result in the claimed invention.

Accordingly, Applicants request that the rejection be withdrawn and the claims allowed.

CONCLUSION

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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Respectfully submitted,

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